

CLAIMS

1. A cyclic maltosylmaltose having a structure of
cyclo(\rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-
5 glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow).

2. A cyclic maltosylmaltose-forming enzyme which has an
activity of forming a cyclic maltosylmaltose having a structure of
cyclo(\rightarrow 6)- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-
glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow) from α -1,4 glucan having
10 a glucose polymerization degree of 3 or higher.

3. The cyclic maltosylmaltose-forming enzyme of claim 2, which
has the following physicochemical properties:

- (1) Molecular weight
72,000 \pm 20,000 daltons on SDS-PAGE;
- 15 (2) Isoelectric point
pI 3.6 \pm 0.5 on isoelectrofocusing using a carrier
ampholyte;
- (3) Optimum temperature
50-55°C when reacted at pH 6.0 for 30 min;
- 20 (4) Optimum pH
5.5 to 6.5 when reacted at 40°C for 30 min;
- (5) Thermal stability
Stable up to the temperature of 30°C when incubated
at pH 6.0 for 60 min;
25 Stable up to the temperature of 50°C when incubated
at pH 6.0 for 60 min in the presence of 1 mM Ca²⁺
ion; and
- (6) pH Stability

Stable in a range of pH 5.0 to 9.0 when incubated
at 4°C for 24 hours.

4. The cyclic maltosylmaltose-forming enzyme of claim 2 or
3, having an amino acid sequence of SEQ ID NO:1 as N-terminal amino acid
5 sequence.

5. The cyclic maltosylmaltose-forming enzyme of any one of
claims 2 to 4, which has an amino acid sequence of SEQ ID NO:2 or an amino
acid sequence having deletion, replacement, or addition of one or more
amino acid residues of SEQ ID NO:2 without altering the enzyme activity.

10 6. The cyclic maltosylmaltose-forming enzyme of any one of
claims 2 to 5, wherein said α -1,4 glucan having a glucose polymerization
degree of 3 or higher is one or more saccharides selected from the group
consisting of maltooligosaccharide, maltodextrin, amyloextrin, amylose,
amylopectin, soluble starch, liquefied starch, gelatinized starch and
15 glycogen.

7. The cyclic maltosylmaltose-forming enzyme of any one of
claims 2 to 6, which is derived from a microorganism.

8. The cyclic maltosylmaltose-forming enzyme of claim 7,
wherein said microorganism belongs to the genus *Arthrobacter*.

20 9. The cyclic maltosylmaltose-forming enzyme of claim 8,
wherein said microorganism belonging to the genus *Arthrobacter* is
Arthrobacter globiformis M6 (International Patent Organism Depository,
National Institute of Advanced Industrial Science and Technology,
Accession No. FERM BP-8448) or a mutant thereof.

25 10. A microorganism capable of producing the cyclic
maltosylmaltose-forming enzyme, which is *Arthrobacter globiformis* M6
(International Patent Organism Depository, National Institute of Advanced
Industrial Science and Technology, Accession No. FERM BP-8448) or a mutant
thereof.

11. A DNA, which encodes the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9.

12. The DNA of claim 11, which comprises a nucleotide sequence of SEQ ID NO:3, a nucleotide sequence having deletion, replacement, or addition of one or more nucleotides of SEQ ID NO:3 without altering the encoded enzyme activity, or complementary nucleotide sequences thereof.

13. The DNA of claim 11 or 12, which is obtainable by replacing one or more nucleotides of SEQ ID NO:3 without altering the amino acid sequence encoded thereby based on the degeneracy of genetic code.

14. The DNA of any one of claims 11 to 13, which is derived from a microorganism of genus *Arthrobacter*.

15. A replicable recombinant DNA, which comprises the DNA of any one of claims 11 to 14 and an autonomously replicable vector.

16. The replicable recombinant DNA of claim 15, wherein said autonomously-replicable vector is a plasmid vector, Bluescript II SK(+).

17. A transformant, which is obtainable by introducing the recombinant DNA of claim 15 or 16 into an appropriate host.

18. The transformant of claim 17, wherein said host is a microorganism of the species *Escherichia coli*.

19. A process for producing the cyclic maltosylmaltose-forming enzyme, comprising the steps of:

culturing a microorganism capable of producing the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9 in a nutrient culture medium; and

collecting the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9 from the resulting culture.

20. The process of claim 19, wherein said microorganism belongs to the genus *Arthrobacter*.

21. The process of claim 20, wherein said microorganism

belonging to the genus *Arthrobacter* is *Arthrobacter globiformis* M6 (International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Accession No. FERM BP-8448) or a mutant thereof.

5 22. A process for producing a recombinant cyclic maltosylmaltose-forming enzyme, comprising the steps of:

 culturing the transformant of claims 17 or 18; and

 collecting the recombinant cyclic maltosylmaltose-forming enzyme from the resulting culture.

10 23. A method for forming a cyclic maltosylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$, comprising a step of allowing the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9 to act on a solution containing $\alpha\text{-1,4}$ glucan having a
15 glucose polymerization degree of 3 or higher.

 24. The method of claim 23, wherein said $\alpha\text{-1,4}$ glucan having a glucose polymerization degree of 3 or higher is one or more saccharides selected from the group consisting of maltooligosaccharide, maltodextrin, amylopectin, amylose, amylopectin, soluble starch, liquefied starch,
20 gelatinized starch and glycogen.

 25. A cyclic maltosylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$ or a saccharide composition comprising the same, which is obtainable by allowing the cyclic
25 maltosylmaltose-forming enzyme of any one of claims 2 to 9 to act on a solution containing $\alpha\text{-1,4}$ glucan having a glucose polymerization degree of 3 or higher.

 26. The cyclic maltosylmaltose or the saccharide composition

comprising the same of claim 25, wherein said α -1,4 glucan having a glucose polymerization degree of 3 or higher is one or more saccharides selected from the group consisting of maltooligosaccharide, maltodextrin, amyloextrin, amylose, amylopectin, soluble starch, liquefied starch, gelatinized starch and glycogen.

27. A cyclic maltosylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$ or a saccharide composition comprising the same, which is obtainable by the steps of:

allowing the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9 to act on a solution containing α -1,4 glucan having a glucose polymerization degree of 3 or higher to form a solution containing the cyclic maltosylmaltose and other saccharides; and

subjecting the resulting solution to a column chromatography using a strongly-acidic cation exchange resin.

28. A saccharide composition comprising a cyclic maltoseylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$, which comprises the cyclic maltosylmaltose in an amount of 1% (w/w) or higher, on a dry solid basis.

29. A cyclic maltosylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$ or a saccharide composition comprising the same, which is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystal, or crystalline solid.

30. A process for producing a cyclic maltosylmaltose having a structure of $\text{cyclo}(\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 6)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4)\text{-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow)$ or a saccharide

composition comprising the same, comprising a step of allowing the cyclic maltosylmaltose-forming enzyme of any one of claims 2 to 9 to act on a solution obtained by gelatinizing and/or liquefying starch.

31. The process of claim 30, where the DE value of said solution
5 obtained by gelatinizing and/or liquefying starch is 20 or lower.

32. The process of claim 30 or 31, comprising the steps of:
allowing the cyclic maltosylmaltose-forming enzyme of any
one of claims 2 to 9 together with isoamylase to act on a solution obtained
by gelatinizing and/or liquefying starch; and
10 optionally, further allowing one or more enzymes selected
from the group consisting of α -amylase, β -amylase, cyclodextrin
glucanotransferase, glucoamylase, and α -glucosidase, to act on the
solution.

33. The process of claim 30 or 31, comprising the steps of:
15 allowing the cyclic maltosylmaltose-forming enzyme of any
one of claims 2 to 9 together with isoamylase to act on a solution obtained
by gelatinizing and/or liquefying starch;
optionally, further allowing one or more enzymes selected
from the group consisting of α -amylase, β -amylase, cyclodextrin
20 glucanotransferase, glucoamylase, and α -glucosidase, to act on the
solution; and

purifying the resultant mixture by one or more methods
selected from the group consisting of fractionation by column
chromatography, separation by membrane, fermentation by a microorganism,
25 and elimination by alkaline treatment.

34. The process of any one of claims 30 to 33, where the product
comprises the cyclic maltosylmaltose in an amount of 1% (w/w) or higher,
on a dry solid basis.

35. The process of any one of claims 30 to 34, where the product is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystal, or crystalline solid.

36. A composition, comprising a cyclic maltosylmaltose having
5 a structure of $\text{cyclo}\{\rightarrow 6\}-\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4\text{)-}\alpha\text{-D-glucopyranosyl-}$
 $\text{(1}\rightarrow 6\text{)-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow 4\text{)-}\alpha\text{-D-glucopyranosyl-(1}\rightarrow \text{)}$ or a saccharide
composition comprising the same.

37. The composition of claim 36, wherein said composition is a food, beverage, cosmetic, or pharmaceutical.

10 38. The composition of claim 36 or 37, which comprises the cyclic maltosylmaltose in an amount of 0.1% (w/w) or higher, on a dry solid basis.